CARBOHYDRATE CONTENT AND OXYGEN UPTAKE IN LARVAE OF RICE STEM BORER, *CHILO SUPPRESSALIS* WALKER

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The rice stem borer, *Chilo suppressalis* WALKER, is one of the most serious insect pests of rice crops in Japan. This species normally has two-brood a year in most parts of the country but it has one-brood a year in the mountainous parts of the Chūgoku District (Yagi 1934, Fukaya 1974, Ishikura 1955). Fukaya (1947) showed that the one-brood zone is an iso-contour area of 300-350 meters from sea level in the mountainous parts of the Chūgoku District in a year with a cool summer.

These broods were determined only by light trap records, and no previous attempt has been made to examine differences in physiology between the one-brood and two-brood rice stem borer.

Many investigators has reported the effect of temperature and photoperiod on the growth and development of this larvae (Harukawa et al. 1931, Inoue and Kamano 1957, Kishino 1969). On the other hand, the hormonal mechanism was investigated for this larval diapause (Fukaya 1957, Fukaya and Mitsuhashi 1957, 1958, Yagi 1975) but there were few investigations of metabolic regulations in this diapausing larvae.

Present investigation was undertaken for obtaining information on the comparative physiology between the one-brood and two-brood larvae.

It has been observed that oxygen uptake and carbohydrate content change very much during diapausing and non-diapausing stages (Wyatt 1967). Carbohydrates which accumulate in diapausing insects are glycogen, glycerol and sorbitol. Glycerol accumulates in diapausing insects (Salt 1959, Takehara and Asahina 1961, Takeda and Hukushima 1962, Sømme 1964, 1965, Mansingh and Smallman 1972, Frankos and Platt 1976) and both sorbitol and glycerol appear in the diapausing egg of the silkworm, *Bombyx mori* (Chino 1957). Glycogen is converted to sorbitol and glycerol at the beginning of diapause and at the termination of diapause, sorbitol and glycerol are reconverted to glycogen in the egg of the silkworm (Chino 1957 a, b, 1958). On the other hand, Hodek and Cerkasov (1961) and Nettles and Betz (1965) found a higher titer of glycogen in diapausing than in reproducing insects. In the rice stem borer, Kamano and Inoue (1955) reported only the content change of water, glycogen and lipid in post-diapausing larvae. They observed that glycogen was changeable.

The present authors examined the comparative physiology between larvae collected from univoltine areas and bivoltine areas under both laboratory and natural conditions. Furthermore, the carbohydrate metabolism of these larvae were investigated.

MATERIALS AND METHODS

Source of insects and rearing

Field collected larvae

Bivoltine larvae (K-larvae)

Most of these diapausing larvae hybernate in rice stems in the winter in Kurashiki city, Okayama prefecture. Thus, the hybernating larvae were collected at Kurashiki and its environs from rice stems in paddy fields.

Univoltine larvae (H-larvae)

Most of these diapausing larvae hybernate in rice stubbles in the Hiru-mountain area of Okayama prefecture. Thus, rice stubbles were gathered from this area in the end of September or beginning of October and were kept in a frame house of our laboratory. These collecting stations were located about 500 meters above sea level.

Laboratory reared larvae

Larvae were reared on budding rice seeds at 25 ℃ under 16 hours light, 8 hours darkness (16L, 8D) and 10 hours light, 14 hours darkness (10L, 14D) photoperiod as described by Satō (1964). The budding rice seeds were exchanged at intervals of 3 to 4 days.

Haemolymph samples

The haemolymph was drained into a glass capillary cooled by ice water through incision in the dorsal cuticle with a fine insect pin. Pooled haemolymph (0.1 ml) was suspended in 0.9 ml of 80 % ethanol, heated at 80 ℃ for 2 minutes, cooled in ice water, and cetrifuged at 3,000 r.p.m. for 5 minutes. Sugar and polyol of the supernatant was analyzed by chromatography.

Extraction of total sugars

After the haemolymph was collected, fat body, digestive organs and the remaining tissues were taken out in saline or Ringer solution. The separate tissues and the whole body of larvae were weighed and homogenized with 5 or 10 ml of 80 % ethanol. From the pooled haemolymph, a 10 μl portion was taken for trehalose estimation and suspended in 5 ml of 80 % ethanol. The homogenates and haemolymph were allowed to stand at 4 ℃ overnight after heating at 80 ℃ for 5 minutes. The clear extracts were obtained by centrifuging at 3,000 r.p.m. for 10 minutes and analyzed for total sugars.

Extraction of glycogen

After extractions of total sugars the ethanol insoluble precipitates from the tissues and whole body were suspended in 5 or 10 ml of 5 % trichloro acetic acid (TCA). The suspsnsions were heated at 100 ℃ for 10 minutes, kept at 4 ℃ for 2 hours after cooling in ice water and centrifuged at 3,000 r.p.m. for 10 minutes. The supernatants were appropriately diluted and analyzed for glycogen.

Quantitative analysis of total sugars and glycogen

Total sugars and glycogen were estimated by the procedure of Yamashita and Hasegawa (1964) employing anthrone as the color reagent with a Hitachi

spectrophotometer (model 139). Anthrone reagent (4 ml) was mixed with 0.5 ml of aqueous sample and heated at 80 ℃ for 30 minutes.

The total sugars in tissues and whole body were expressed as glucose equivalents, and the sugar in haemolymph was expressed as trehalose equivalents.

Determination of sugar and polyol

Sugar and polyol were separated. An aliquot of the supernatant was developed on fillter paper (Tōyō filter paper No. 51A) using butanol: pyridine: water (6: 4: 3) and phenol: water (5 : 1) as solvents and on a silica gel G thin layer plate using butanol: acetone: water (4: 5: 1) and chloroform: methanol (6: 4).

For detection and identification of sugar and polyol on paper chromatography, silver nitrate reagent was used after chromatography (Trevelyan 1950). On thin layer chromatography, the anthrone reagent used to estimate total sugars and glycogen was sprayed on a thin layer plate and heated at 100 ℃ for 10 minutes.

Quantitative analysis of glycerol

After completion of development, glycerol was eluted from filter paper with 5 ml of 80 % ethanol. The quantity of glycerol contained in the eluent was determined by the colorimetric method of Fletcher (1968) with some modifications.

Reagent

Sodium metaperiodate (0.025 M) was used in 1.0 N acetic acid. The working solution was diluted to 12 ml of sodium metaperiodate with 88 ml of 0.1 N acetic acid. For acetyl acetone, 0.75 ml of 2,4-pentanedione was dissolved in 100 ml of 2 M ammonium acetate.

The working sodium metaperiodate solution (1 ml) was added to 1 ml of 80 % ethanol eluent. The solution mixed and incubated for 10 minutes at room temperature. Acethyl acetone (2 ml) was then added, the solution mixed, and incubated at 37 ℃ for 40 minutes. After cooling the color was read with a spectrophotometer at an optimum density at 415 nm.

Oxygen uptake

The diapausing larvae were kept at 25 ℃ overnight before measurement of oxygen uptake. The oxygen uptake was measured by a Warburg manometer at 25 ℃ by usual procedures.

RESULTS

Laboratory-reared larvae

Both H-larvae and K-larvae entered diapause under short day length (10L, 14D) at 25 ℃ and non-diapausing larvae were observed under long day length (16L, 8D) as reported by Kishino (1969).

Total sugars and glycogen contents

The changes in total sugars and glycogen content in whole body are re-

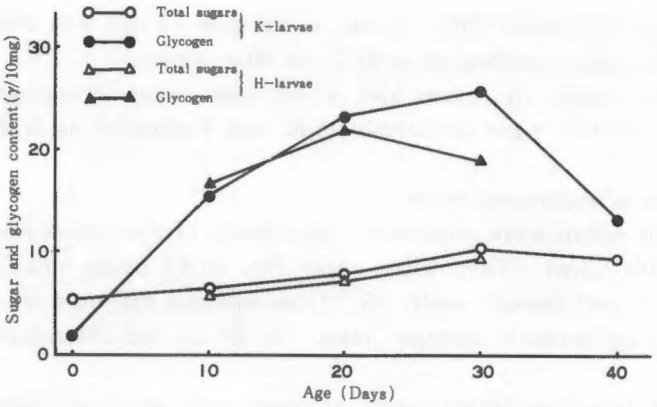

Fig. 1. Sugar and glycogen content of larvae reared at 25°C under 16L, 8D photoperiod.

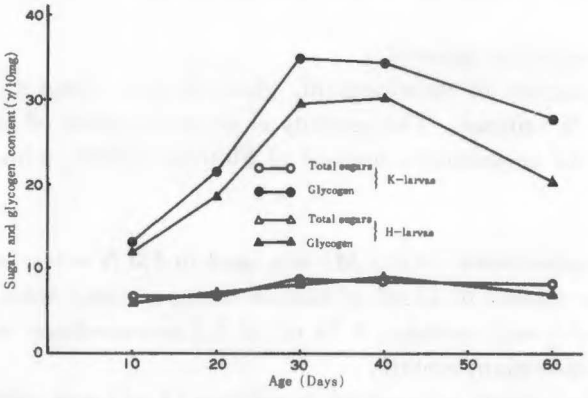

Fig. 2. Sugar and glycogen content of larvae reared at 25°C under 10L, 14D photoperiod.

ported on non-diapausing larvae under LD photoperiod (Fig. 1) and SD photoperiod (Fig. 2). Total sugars content in larvae, calculated on a weight basis, increased slightly during subsequent growth of non-diapausing larvae, but on the contrary, these measures were at constant levels in diapausing larvae. Glycogen content in larvae, caluculated on a weight basis, increased during subsequent growth of non-diapausing larvae as in diapausing larvae, but the glycogen content in diapausing larvae increased to a higher level than in non-diapausing larvae. Glycogen content decreased in non-dipausing H-larvae at 30 days, but in non-diapausing K-larvae at 40 days. Glycogen content continued at high levels in both diapausing K-and H-larvae.

Sugar in haemolymph

The only free sugar detected in haemolymph was trehalose and other sugars and glycogen were rarely detected (Figs. 12, 13, 14). The content of trehalose

in haemolymph increased gradually during subsequent growth (Fig. 3). No significant difference in haemolymph trehalose content was found between H-larvae and K-larvae.

Oxygen uptake

Oxygen uptake was estimated for several instar larvae, and these results are shown in Fig. 4. Oxygen uptake, calculated on a weight basis, decreased progressively with growth under both LD and SD photoperiods. Non-diapausing larvae consumed slightly higher amounts of oxygen than diapausing H-larvae but no significant difference in respiration was found between non-diapausing and diapausing K-larvae. H-larvae consumed higher amounts of oxygen than K-larvae during larval growth.

Field-collected larvae

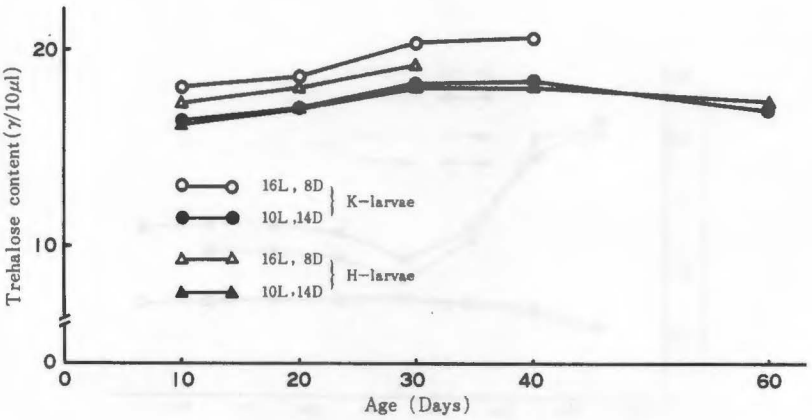

Fig. 3. Trehalose content in haemolymph of larvae reared at 25°C.

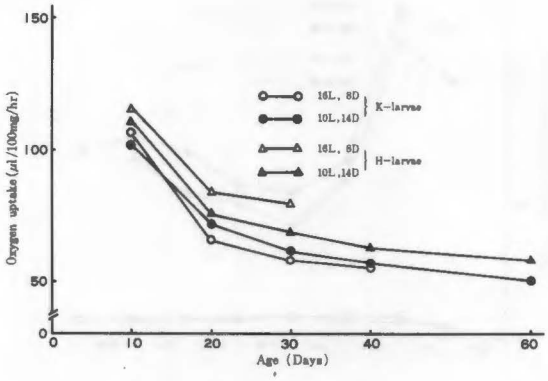

Fig. 4. Oxygen uptake by larvae reared at 25°C under 16L, 8D photoperiod.

It is known that K-larvae in fields may enter diapause in December and the diapause break at the middle of February, and H-larvae may enter diapause in November and the diapause break at the middle of February.

Total sugars and glycogen content

Fig. 5 shows the changes in total sugars and glycogen content in the whole body of hybernating larvae. The total sugars content in the whole body, caluclated on a weight basis, increased slightly during pre-diapausing stage and thereafter, was at an approximately constant level during the post-diapausing stage. On the other hand, the amount of glycogen in the whole body increased during the pre-diapausing stage and decreased during the diapausing stage. Thereafter, the amount temporarily increased during the post-diapausing stage and decreased slightly again before pupation.

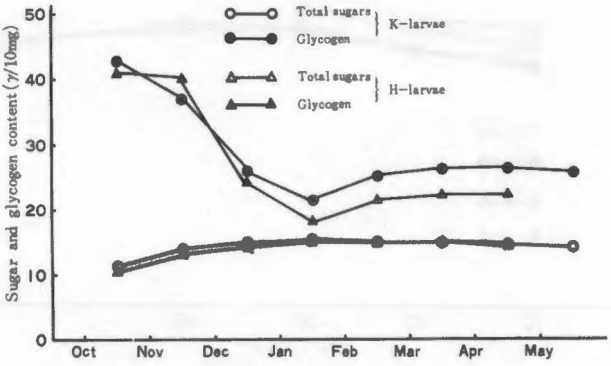

Fig. 5. Sugar and glycogen content in whole body of hybernating larvae collected in fields.

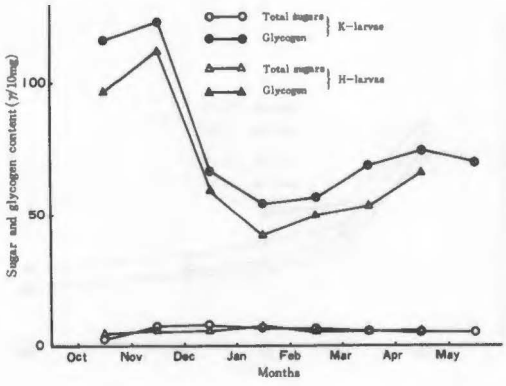

Fig. 6. Sugar and glycogen content in fat body of hybernating larvae collected in fields.

Changes are reported on total sugars content in fat body(Fig. 6), digestive organs (Fig. 7) and the remaining tissues (Fig. 8). Total sugars contents in the fat body and the remaining tissues were at approximately constant levels in hybernating larvae, but the content in the digestive organs decreased silghtly. The amount of glycogen in each tissues decreased gradually during the diapausing stage as in the whole body (Fig. 5). The glycogen content in the fat body increased during the pre-diapausing stage and decreased very much during the diapausing stage. Then, the content increased again during the post-diapausing stage but did not increase at the initial level and decreased before pupation. The content of glycogen in the digestive organs and the remaining tissues changed as in the fat body. Glycogen in the remaining tissues decreased again before pupation as in the fat body but the content in the digestive organs did not

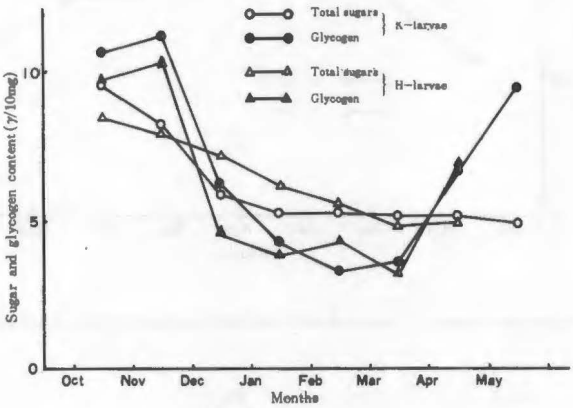

Fig. 7. Sugar and glycogen content in digestive organs of hybernating larvae collected in fields.

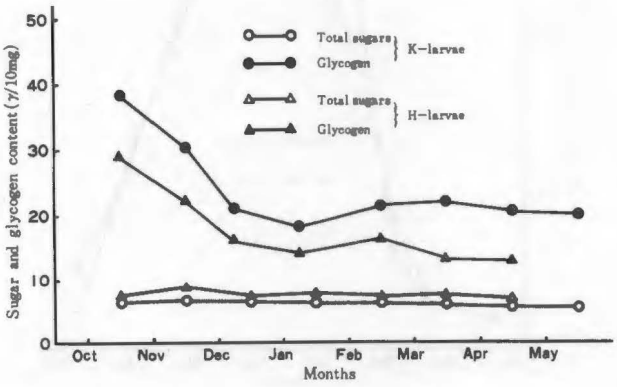

Fig. 8. Sugar and glycogen content in the remaining tissues of hybernating larvae collected in fields.

decrease. No significant differences were found in free sugars content between K-larvae and H-larvae during hybernation.

Sugar in haemolymph

The detected free sugar in haemolymph was trehalose, and other sugars were rarely detected in hybernating larvae as in laboratory-reared larvae (Figs. 12 – 1, 13, 14). Trehalose content in haemolymph increased during the diapausing stage and decreased at the initial level when diapause terminated (Fig. 9).

Amount of glycerol in haemolymph and tissues

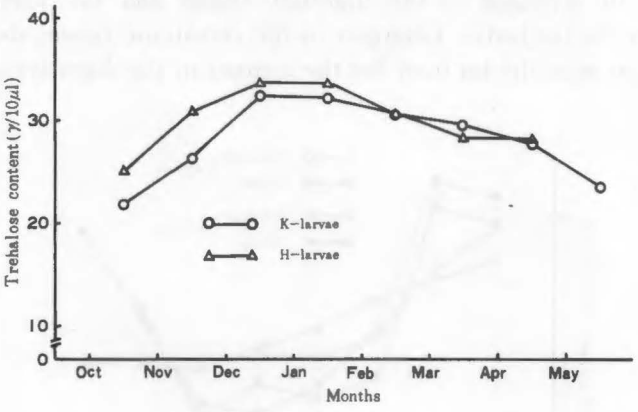

Fig. 9. Trehalose content in haemolymph of hybernating larvae collected in fields.

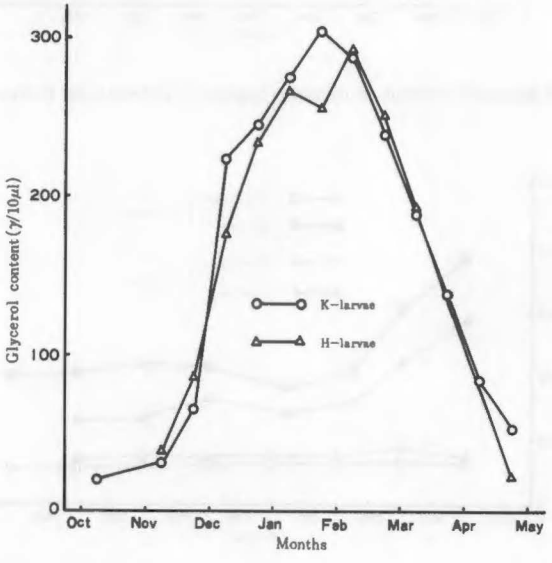

Fig. 10. Glycerol content in haemolymph of hybernating larvae collected in fields.

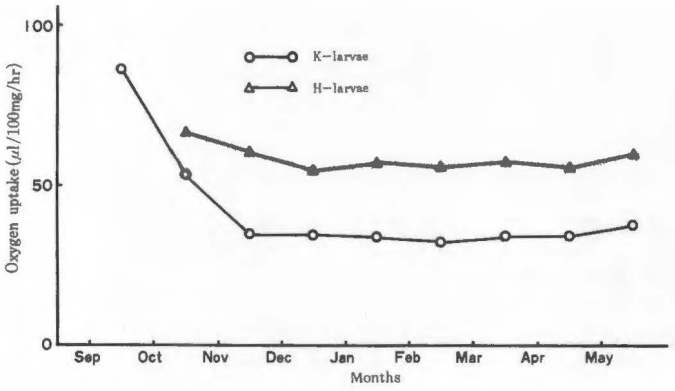

Fig. 11. Oxygen uptake by hybernating larvae collected in fields.

The amount of glycerol in haemolymph increased very much during diapausing stage (Fig. 10). The glycerol content was at the highest level in January to February, but decreased very much during the post-diapausing stages. Table 1 shows the amount of glycerol in each tissue. The amounts of glycerol in haemolymph and digestive organs on per weight of tissue of diapausing larvae, were at higher levels than in fat body and the remaining tissues. On the other hand, in the remaining tissues of the post-diapausing larvae, the amount did not decrease as in other tissues. Sorbitol could not be detected (Figs. 13,14,). In diapausing larvae at 25 ℃ under 10L, 14D glycerol could not be detected (Figs. 12,14).

Oxygen uptake

Fig. 11 shows the changes in oxygen uptake in hybernating larvae. The oxygen uptake in K-larvae on a weight basis, decreased in September to November, thereafter, maintained constant level and increased slightly in May. On the other hand, the oxygen uptake in H-larvae maintained approximately constant level in October. The amount of oxygen uptake in H-larvae was higher than in K-larvae.

TABLE 1

Glycerol content in tissues of K-larvae collected in fields

Tissues	Collecting date	
	January 28 (γ/10 mg tissue)	March 12 (γ/10 mg tissue)
Fat body	34.6	11.2
Digestive organs	177.2	52.4
Remaining tissues	50.2	49.5

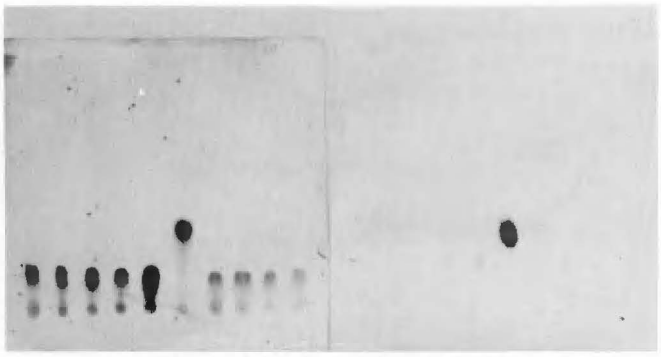

Fig. 12–1. Sugar constitution in haemplymph of larvae reared at 25 ℃ under 16L, 8D and 10L, 14D.

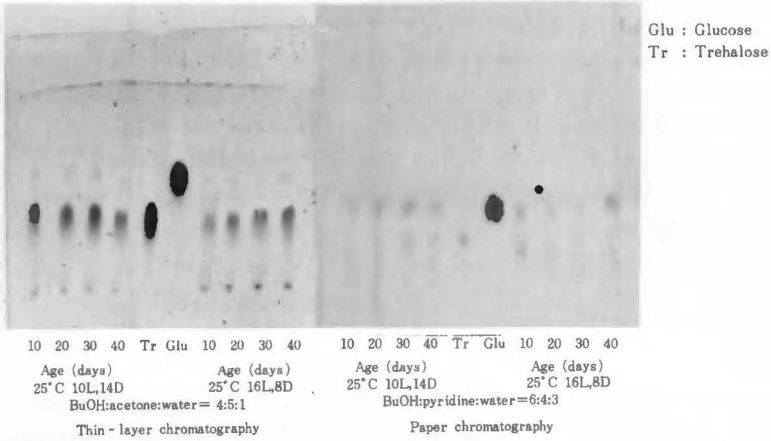

Fig. 12–2. Sugar constitution in the whole body of larvae reared at 25℃ under 16L, 8D and 10L, 14D.

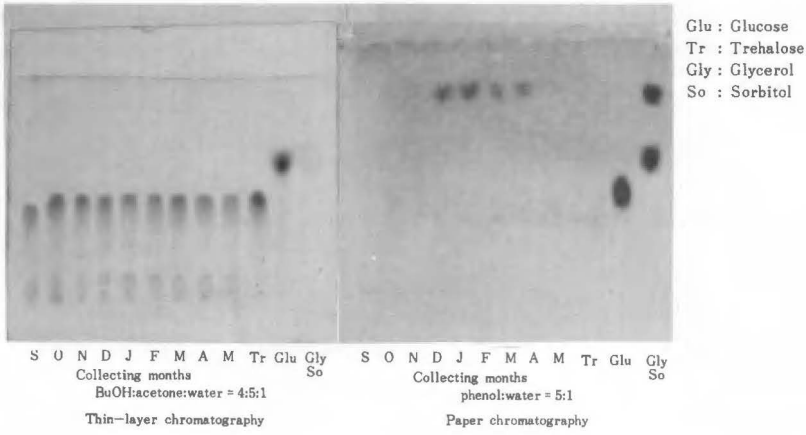

Fig. 13. Seasonal changes in sugar constitution in haemolymph of hybernating larvae.

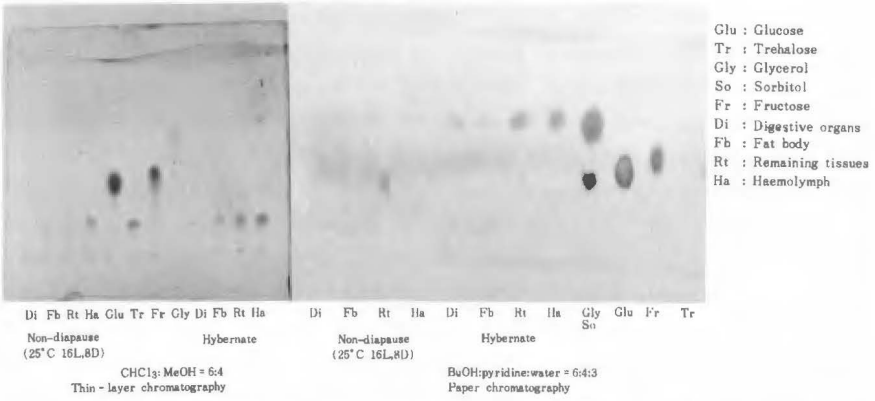

Fig. 14. Sugar constitution in each tissue of non-diapausing and hybernating larvae.

DISCUSSION

The mean oxygen uptake in K-larvae of the rice stem borer, *Chilo suppressalis* WALKER, decreased from September to November, thereafter, the oxygen level was at a low level and increased slightly before pupation. Fukaya (1949) reported similar changes in the rate of oxygen uptake in diapausing larvae of the same species collected at Kurashiki. On the other hand, the oxygen uptake of H-larvae was already at a constant level in October. This result indicated H-larvae in field may have already entered diapause. That is, the season when H-larvae enter diapause may be earlier than in K-larvae. Fukuda (1937) and Ishikura (1939) reported the rate of oxygen uptake per unit weight correlated with the weight of the diapausing larvae of the rice stem borer, and the oxygen uptake rate of light weight larvae was higher than that of heavy weight larvae. As the weight of both H-larvae collected from the field and reared in the laboratory were less than of K-larvae, it was suggested that the H-larvae rate of oxygen uptake was higher than that of K-larvae. In comparing non-diapausing and diapausing larvae reared in the laboratory, H-larvae oxygen uptake in the non-diapausing stage was slightly higher than that of diapausing larvae, but in K-larvae the oxygen uptake of diapausing larvae was rather higher level than non-diapausing larvae. However, no significant differences in respiration were found between non-diapausing and diapausing larvae. A slightly difference in respiration was also found between non-diapausing fully mature larvae reared in the laboratory and hybernating larvae collected from fields. These results may allow the statement that the respiratory metabolism rate is maintained at a high level during hybernation. Furthermore, in view of this observation, some hybernating larvae of the rice stem borer continued to feed in rice stems during the diapausing stage (Fukaya 1961). It may thus be possible to say that the respiratory metabolism rate of these larvae differ from that of other diapausing insects (Chino 1958, Mansingh and Steele 1964).

Total sugars contents were at approximately constant levels but glycogen content increased during subsequent growth in both diapausing and non-diapausing larvae reared in the laboratory. Hodek and Cerkasov (1961) found glycogen higher content in diapausing than that in reproducing forms of *Coccinella septempunctata*. Nettles and Betz (1965) reported the similar phenomenon in the boll weevil, *Anthonomus grandis* BOHEMAN. Similar results were also obtained in the present study. On the other hand, Zaluska (1959) reported that the amount of glycogen in the silk worm, *B. mori*, decreased during metamorphosis. The glycogen content in the non-diapausing H-larvae decreased at 20 to 30 days and in K-larvae at 30 to 40 days. That is to say, in the same condition the H-larval period may be shorter compared with the K-larval period. Furthermore, glycogen contents in the whole body and fat body of hybernating larvae collected from fields decreased slightly in May. The glycogen contents were erroneouslly evaluated in previous observations.

Glycerol accumulates considerably in haemolymph of hybernating larvae collected from fields. The glycerol content in haemolymph of H-larvae was temporarilly decreased at the end of January. However, this is because the small number of H-larvae used to estimate glycerol content caused an error of measurement. If many more H-larvae were used for estimation, the glycerol content in H-larvae may be similar to the K-larvae rate. Chino (1957 a, b, 1958) found that in diapausing eggs of the silk worm, *B. mori*, glycogen was converted into sorbitol and glycerol, and glycogen was resynthesized from these polyhydric alcohol when diapause terminated. Wyatt and Meyer (1959), Takehara and Asahina (1960) and Takeda and Hukushima (1961) also found that glycerol accumulated in the blood of diapausing insects. Furthermore, they suggested that glycerol may be a product of glycogen. It was also seen in the present study that the formation of glycerol in haemolymph with the onset of diapause was associated with a decline in glycogen content in fat body, and when diapause terminated, the amount of glycerol in haemolymph began to decreased progressively, while the amount of glycogen in fat body steadily increased. Kamano and Inoue (1955) also reported that glycogen increased in the first 5 days, and thereafter, decreased when post-diapausing larvae of the rice stem borer were transferred to 25 ℃. It can be also anticipated that the temporary increase in glycogen maybe produced from glycerol.

Salt (1957, 1958), Sømme (1964, 1965) and Asahina (1969) found high levels of glycerol which have great effects upon both the supercooling points and likelihood of tissue injury when freezing occurs. The air temperature of the Hiru-mountain is lower than that of Kurashiki in winter, but the glycerol contents in haemolymph of diapausing larvae were at similar levels in larvae from both sites. This may have been caused by the transfer from an univotine area to bivoltine area. However, both these diapausing larvae could survive freezing at temperatures below −20 ℃. Mansingh and Smallman (1972) and Frankos and Platt (1975) reported glycerol accumulation may not begin until insects are sub-

jected to colder temperature. That is to say, in diapausing larvae of the rice stem borer, glycerol may also accumulate under colder temperature.

Glycerol contents in the fat body and digestive organs decreased during the post-diapausing stage, on the contrary the content in the remaining tissues did not decrease. These results may allow the tentative conclusion that glycerol is a metabolite in the fat body and digestive organs, but this conclusion needs to be investigated in further detail.

SUMMARY

Non-diapausing and diapausing larvae of the rice stem borer, *Chilo suppressalis* WALKER, from univoltine and bivoltine area were examined for changes in carbohydrate content, oxygen uptake and carbohydrate metabolism.

A large difference was not observed between univoltine and bivoltine larvae in carbohydrate content and oxygen uptake. However, in the laboratory, the univoltine larval period was shorter than the bivoltine larval period, and the season when the univoltine larvae entered diapause was earlier than bivoltine larvae in fields. Thus, earlier changes in carbohydrate content and oxygen uptake occurred in univoltine larvae compared with bivoltine larvae.

A large difference in carbohydrate metabolism was observed in the diapausing larvae under a short day photoperiod at 25 ℃ and hybernating larvae in fields. That is to say, glycerol in the diapausing larvae collected in fields was high level, but glycerol could not be detected in the diapausing larvae under a short day photoperiod at 25 ℃.

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